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Human immunodeficiency virus bDNA assay for pediatric cases

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SUMMARY

Techniques to quantify plasma HIV-1 RNA viral load (VL) are commercially available, and they are adequate for monitoring adults infected by HIV and treated with antiretroviral drugs. Little experience on HIV VL has been reported in pediatric cases. In Argentina, the evaluation of several assays for VL in pediatrics are now being considered. To evaluate the pediatric protocol for bDNA assay in HIV-infected children, 25 samples from HIV-infected children (according to CDC criteria for pediatric AIDS) were analyzed by using Quantiplex HIV RNA 2.0 Assay (Chiron Corporation) following the manufacturer's recommendations in a protocol that uses 50 µl of patient's plasma (sensitivity: 10,000 copies/ml). When HIV-RNA was not detected, samples were run with the 1 ml standard bDNA protocol (sensitivity: 500 HIV-RNA c/ml). Nine samples belonged to infants under 12 months of age (group A) and 16 were over 12 months (group B). All infants under one year of age had high HIV-RNA copies in plasma. VL ranged from 30,800 to 2,560,000 RNA copies/ml (median = 362,000 c/ml) for group A and < 10,000 to 554,600 c/ml (median = < 10,000) for group B. Only 25% of children in group B had detectable HIV-RNA. By using the standard test of quantification, none of the patients had non detectable HIV-RNA, ranging between 950 and 226,200 c/ml for group B (median = 23,300 RNA c/ml). The suggested pediatric protocol could be useful in children under 12 months of age, but 1 ml standard protocol must be used for older children. Samples with undetectable results from children under one year of age should be repeated using the standard protocol.

Key words: viral load, pediatric protocols, HIV/AIDS, RNA quantitation

RESUMEN

Ensayo de bDNA para el virus de la immunodeficiencia humana en casos pediátricos. Existen varias técnicas de disposición comercial para la cuantificación de virus con genoma ARN. Se utilizan muy especialmente para medir la carga viral de adultos infectados con HIV. Sin embargo, la experiencia en carga viral de HIV en pediatría sigue siendo escasa. Actualmente se está evaluando la factibilidad de uso de cada una de las técnicas. Se evaluó el protocolo pediátrico de la técnica bDNA para medir la carga viral del HIV. Se analizaron 25 muestras de niños infectados con HIV (infectados de acuerdo al criterio de SIDA pediátrico del CDC, Centro de Control de Enfermedades de Estados Unidos). Se utilizó un equipo de bDNA Quantiplex HIV RNA 2.0 Assay de Chiron Corporation. Se siguieron las recomendaciones de la compañía, utilizando 50 µl de plasma (sensibilidad: 10.000 copias/ml). Nueve muestras de plasma fueron obtenidas de niños de menos de 12 meses de edad (grupo A) y 16 muestras de niños mayores de 12 meses (grupo B). Todos los niños menores del año de edad tuvieron altos niveles de ARN en plasma, variando entre 30.800 y 2.560.000 copias/ml (mediana: 362.000). Los niños del grupo B, en cambio, mostraron bajos valores de carga viral, entre < 10.000 y 554.000 copias/ml (mediana: < 10.000). En el grupo B, sólo el 25% de los pacientes tuvieron carga viral detectable. Utilizando el ensayo estándar de bDNA (que utiliza 1 ml de plasma), ninguna de las muestras tuvo niveles no detectables de ARN (rango: 950-226.200, mediana: 23.300 copias/ml). El protocolo pediátrico sugerido puede ser de utilidad para medir la carga viral en niños menores de 12 meses de edad. Sin embargo, para los pacientes con más de un año de edad es preferible utilizar el protocolo estándar. Muestras que no presentan niveles detectables de ARN de HIV en niños menores de un año, podrían aparecer por lo que en estos casos se recomienda repetir la muestra siguiendo el protocolo estándar.

Palabras claves: carga viral, cuantificación de ARN, HIV/SIDA, SIDA en pediatría

INTRODUCTION

Prospective studies have reported HIV vertical transmission in 13 to 39% of infants born to HIV-infected women (7, 8, 20). Pediatric AIDS is the result of a multifactorial process of transmission. Viral, genetic, immune and clinical factors in both the mother and the infant play potential roles (2). AIDS Clinical Trials Group (ACTG) study 076 by Connor *et al* (5) has shown that perinatal transmission could be reduced by approximately 70% by the use of zidovudine (ZDV) when given to HIV-infected drug-naive women during gestation and delivery, and for 6 weeks to the infant following birth. Nowadays, the results of different studies have confirmed the concept that perinatal transmission can be significantly reduced by the use of antiretroviral therapy, and trials of combined antiretroviral therapy are in progress (15, 19). The aim of these trials is to reduce viral load to assess the feasibility of permanent suppression or eradication of HIV. Techniques to quantify plasma HIV-1 RNA are available (12, 16, 22, 23), and they are suitable in monitoring adults and children infected by HIV and treated with antiretroviral drugs. In Argentina, adults with multiple retroviral therapies are being followed since 1996 by using any of the three commercially available methodologies for quantitation of HIV-1 RNA in plasma (Amplicor HIV-1 Monitor test, Roche Diagnostic Systems, USA; NASBA HIV-1 RNA QT, Organon Teknika, Holland; and Quantiplex HIV-1 RNA assay, Chiron Corporation, USA). Little experience on viral load in Latinamerica has been reported specially among infants. Herein, results are presented which were obtained in 1997 from 25 Argentine infected children born to HIV-1 positive mothers using bDNA technology by following the protocol for pediatric cases recommended by Chiron Corporation.

MATERIALS AND METHODS

Plasma samples from 25 children (age 0-96 months) born to HIV-1 positive mothers undergoing different clinical stages were analyzed by using the bDNA methodology (Quantiplex HIV-1 RNA 2.0 assay, Chiron Corporation, USA) following the pediatric protocol recommended by the manufac-turer. This protocol uses only 50 µl of plasma (in duplicates) although the detection limit is decreased by tenfold. Children were considered HIV infected according to CDC criteria for pediatric AIDS (3). Children were divided into two groups. **Group A:** Nine infants under 12 months of age (range: 1 to 8 months), three of these children followed complete protocol ACTG 076. No other treatment was used except for one infant who started double therapy with ZDV and ddI at 5 months of age. **Group B:** 16 children older than 12 months of age (range: 13 to 96 months), 7 of these children were under antiretroviral treatment.

A second plasma sample, was obtained 2-6 months later from 6 children of group A. Fifty µl of plasma in duplicates were used for each sample. This assay has a quantitation limit of 10,000 RNA copies/ml.

Samples that were under the detection values of the assay were run with the standard bDNA method using 1 ml of plasma, in duplicates (sensitivity: 500 HIV-1 RNA copies/ml). Plasma was previously centrifuged at 23500 x g during 1 h. Samples were resuspended in a lysis buffer with target probes complementary to the HIV-1 pol gene sequences. No extraction step was carried out. Quantiplex assay is based on serial hybridization with DNA probes and one of the probes is a branched DNA molecule that serves as amplifier (22). The capture of the HIV-1 RNA is performed overnight at 53 °C in capture well plates. The amplifier (bDNA) contains alkaline phosphatase that reacts with the chemiluminiscent substrate (dioxietane) and light emission is measured in a luminometer. The computer program calculated the amount of HIV-1 RNA in the sample by reference to a standard curve run in parallel with the specimens.

RESULTS

Viral load values obtained with the pediatric protocol of Quantiplex using 50 µl of plasma are shown in Table 1. For group A, values ranged from 30,800 to 2,560,000 HIV RNA copies/ml with a median of 362,000. All of these children had high copy numbers of HIV RNA in plasma, with a 100% sensitivity for the methodology employed. Values

Table 1. Viral load values in 25 HIV infected children

	Patient	Age (months)	Viral load (RNA-copies/ml)	Antiretroviral treatment after ACTG 076 protocol
	1	1	58980	No
	2	2	2560000	ZDV-ddI
	3	3	1152000	No
	4	3	467200	No
Group A	5	3	195480	No
	6	4	107440	No
	7	5	30800	No
	8	8	362000	No
	9	9	625600	No
	10	13	< 10000	No
	11	20	< 10000	No
	12	27	< 10000	ZDV-ddI
	13	42	542200	ZDV-ddI
	14	43	45740	ZDV-ddI
	15	53	< 10000	No
Group B	16	54	< 10000	No
	17	60	< 10000	3TC-d4T
	18	61	< 10000	3TC-d4T
	19	64	< 10000	No
	20	66	< 10000	ZDV-ddI
	21	70	554600	No
	22	72	< 10000	No
	23	76	< 10000	No
	24	84	< 10000	No
	25	96	345200	ZDV-3TC

Sensitivity of the pediatric protocol: 10,000 ARN copies/ml

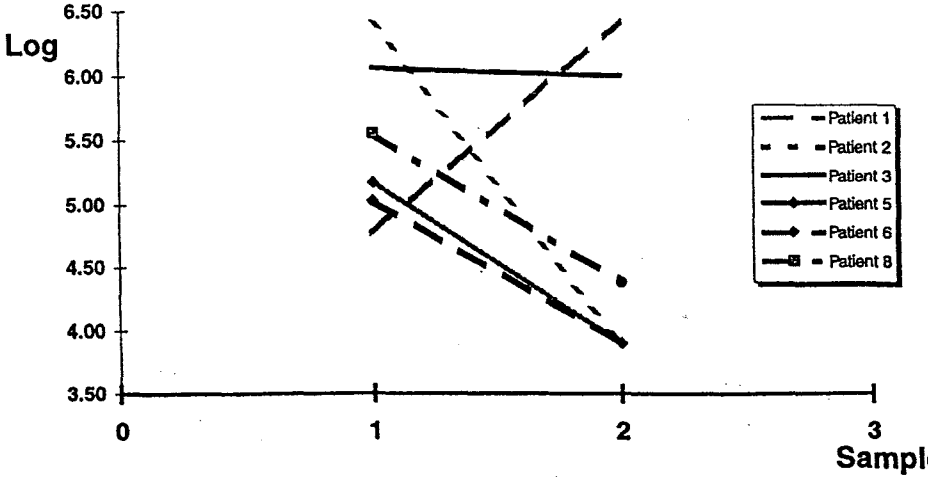

Figure 1. Viral load in consecutive plasma samples from HIV infected children. Patient 1: first sample during ZDV therapy Protocol ACTG 076; patient 2: second sample after starting double therapy (ZDV+ddI).

Table 2. Comparison of viral load values between the standard and the pediatric protocol for bDNA assay

	Patient	Age (months)	Pediatric protocol (RNA-copies/ml)	Standard protocol (RNA-copies/ml)
Group B	10	13	< 10000	48000
	11	20	< 10000	45000
	12	27	< 10000	23300
	13	42	542200	ND
	14	43	45740	120600
	15	53	< 10000	29580
	16	54	< 10000	11500
	17	60	< 10000	950
	18	61	< 10000	30500
	19	64	< 10000	ND
	20	66	< 10000	1067
	21	70	554600	38800
	22	72	< 10000	12520
	23	76	< 10000	15630
	24	84	< 10000	18490
	25	96	345200	226200

ND: not done. Sensitivity of the pediatric protocol: 10,000 ARN copies/ml. Sensitivity of the standard protocol: 500 ARN copies/ml.

for group B were detectable (> 10,000 copies per ml) in only 25% of the children. The median value for this group was < 10,000 ranging from < 10,000 to 554,600 RNA copies/ml. Eleven out of 12 plasma samples with non-detectable HIV RNA and 3 out of 4 with detectable HIV RNA were run again with the standard protocol of Quantiplex and values ranged between 950 and 226,200 RNA copies/ml with a median value of 23,300 copies/ml (Table 2).

Figure 1 shows viral load values for two consecutive samples of 6 children belonging to group A. Two of these children were receiving antiretroviral therapy: patient 1 at the time of the first bleeding and patient 2 during the second bleeding. Thus, the expected results of up-regulation or down-regulation of viral replication, respectively, occurred. Of the other 4 children, 3 lowered the HIV RNA in plasma significantly (more than 0,5 log) and 1 kept the viral load value. None of them were under antiretroviral treatment at the time of obtention of their samples.

DISCUSSION

Different assays to measure viral load have been used to define viral replication kinetics (4, 9, 24), to assess antiretroviral activity (11, 21) or drug resistance (13) and to study vertical transmission (6, 25).

The identification of infants at risk, the performance of an early diagnosis and the assessment of a good prognosis for disease progression are very important points in the management of antiretroviral therapy for HIV infection. Prognostic markers such as viral load, SI - NSI viruses or slow/low - rapid/high strains are as useful in pediatrics as well as in adults infected with HIV-1. A multicenter study conducted with 267 HIV-1 infected children has associated high viral load values during the first week of life with a higher risk of the early and severe form of HIV infection, thus showing the importance of this parameter as basis for early therapeutic intervention (14). Another study in 64 children showed that rapid/

high-SI viruses were isolated from infants who had higher HIV RNA levels in plasma compared to those children with slow/low-NSI viruses; the latter showed a slower progression to AIDS (17). This type of studies are possible since very specific, sensitive and reproducible methodologies are available to quantify HIV-1 RNA (12, 16, 22, 23). However, the determination of viral load in children may result difficult since it is not always possible to obtain the proper sample, especially regarding the amount of blood withdrawn. However, protocols tending to use a few µl of plasma have been developed, but they can cause serious failures in the evaluation of the results (26). They may also show lower sensitivity or reproducibility (10).

This report describes the use of the pediatric protocol of the bDNA assay recommended by Chiron Corporation with 50 µl of plasma sample and compares it with the 1 ml standard protocol. Chiron Corporation suggested this protocol since correlation with quantitation in the 1 ml format was found to be extremely closé (r2: 0,98) (Yeghiazarian T, personal communication). Nevertheless, this conclusion was not at all coincident with results presented here for children over 12 months of age (r2: 0,21). This could be attributed to the fact that viral load was close to limiting dilution of the nucleic acids for the proper detection and quantitation of free HIV virions in 50 µl of plasma. This might imply that children with non detectable viral load values could be exposed to be mismanaged with the perils of not being treated with antiretroviral drugs or even, of not being recognized the existence of earlier drug resistance. This study also shows that children under one year of age normally have very high viral loads and this is in accordance with Naver *et al* (18) since they found that the peak of viral load is between 1.5 and 3 months of age and then decreases in children up to 8 years old. Results reported herein show that the suggested pediatric protocol can be useful in children under 12 months of age. However, if undetectable viral load is registered in samples from those children under 1 year old, it is recommended that the assay be repeated with the 1 ml standard protocol.

On the other hand, PCR assays have proved to end in a high proportion of false-negative results (1). Even though we have tested only a small group of children, the lack of false negative results from children in the first months of life suggests that bDNA can also be of great help in the diagnosis of pediatric HIV infection.

Moreover, when 2 consecutive samples from the same patient were processed significant differences between viral load values were obtained, emphasizing that quantitation of HIV-RNA can be considered a good marker just as in HIV infected adults, even when using the 50 µl pediatric protocol, for the prognosis of disease progression or the management of antiretroviral therapy.

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